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# UCC

**University College Cork, Ireland**  
Coláiste na hOllscoile Corcaigh

1 **Ecological importance of cross-feeding of the intermediate metabolite 1,2-**  
2 **propanediol between bacterial gut symbionts**

3

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16 Running Head: Cross-feeding of gut symbionts through 1,2-propanediol

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20 **Abstract**

21 Cross-feeding based on the metabolite 1,2-propanediol has been proposed to  
22 have an important role in the establishment of trophic interactions among gut symbionts,  
23 but its ecological importance has not been empirically established. Here, we show that  
24 *in vitro* growth of *Lactobacillus reuteri* ATCC PTA 6475 is enhanced through 1,2-  
25 propanediol produced by *Bifidobacterium breve* UCC2003 and *Escherichia coli* MG1655  
26 from the metabolization of fucose and rhamnose, respectively. Work with isogenic  
27 mutants showed that the trophic interaction is dependent on the *pduCDE* operon in *L.*  
28 *reuteri*, which encodes for the ability to use 1,2-propanediol, and the L-fucose permease  
29 (*fucP*) gene in *B. breve*, which is required for 1,2-propanediol formation from fucose.  
30 Experiments in gnotobiotic mice revealed that, although the *pduCDE* operon bestows a  
31 fitness burden on *L. reuteri* ATCC PTA 6475 in the mouse digestive tract, the ecological  
32 performance of the strain was enhanced in the presence of *B. breve* UCC2003 and the  
33 mucus-degrading species *Bifidobacterium bifidum*. The use of the respective *pduCDE*  
34 and *fucP* mutants of *L. reuteri* and *B. breve* in the mouse experiments indicated that the  
35 trophic interaction was specifically based on 1,2-propanediol. Overall, our work  
36 established the ecological importance of cross-feeding relationships based on 1,2-  
37 propanediol for the fitness of a bacterial symbiont in the vertebrate gut.

38

39

40 **Importance**

41 Through experiments in gnotobiotic mice that employed isogenic mutants of  
42 bacterial strains that produce (*Bifidobacterium breve*) and utilize (*Lactobacillus reuteri*)  
43 1,2-propanediol, this study provides mechanistic insight into the ecological ramifications  
44 of a trophic interaction between gut symbionts. The findings improve our understanding  
45 on how cross-feeding influences the competitive fitness of *L. reuteri* in the vertebrate gut  
46 and revealed a putative selective force that shaped the evolution of the species. The  
47 findings are relevant as they provide a basis to design rational microbial-based  
48 strategies to modulate gut ecosystems, which could employ mixtures of bacterial strains  
49 that establish trophic interactions or a personalized approach based on the ability of a  
50 resident microbiota to provide resources for the incoming microbe.

51

## 52 Introduction

53 The gut microbiota is a complex microbial community whose members form  
54 interdependent trophic relationships that determine the ecology of bacterial populations  
55 and their interplay with the host (1). One such interaction involves the exchange of  
56 products between microbes, otherwise known as cross-feeding (2). Cross-feeding of  
57 nutrients is central to the fermentative degradation of non-digestible dietary fibres and  
58 host-derived substrates, such as mucin or human milk oligosaccharides (HMO) (3-10).  
59 For example, glycans released from the partial degradation of HMO are utilized by other  
60 bifidobacterial species (11), and certain bifidobacteria and *Akkermansia muciniphila*  
61 liberate glycans from host mucins that are utilized by other inhabitants of the gut (6, 12).  
62 Similarly, *Ruminococcus bromii* releases carbohydrates from resistant starch  
63 fermentations into the gut environment (13). These interactions ultimately lead to the  
64 formation of dynamic metabolic networks essential in the ecology of the gut microbiome  
65 and the production of short chain fatty acids (SCFA), which are a key ecosystem service  
66 that benefits the host (14, 15).

67 Trophic interactions can also result from the exchange of metabolic end-products  
68 derived from fermentation processes. Hydrogenotrophic microbes utilize the molecular  
69 hydrogen produced by several fermentative organisms, a process important to maintain  
70 energy flux (16). In addition, cross-feeding based on intermediary metabolites such as  
71 lactate, acetate, succinate, and 1,2-propanediol is important for the production of SCFA  
72 (5). In the gut, 1,2-propanediol results from the microbial fermentation of deoxyhexose  
73 sugars that originate from the hydrolyzation of dietary fibre, fucosylated HMOs, and host  
74 mucins, such as rhamnose and fucose (17, 18). Several gut commensal bacteria (i.e.

75 *Bacteroides thetaiotaomicron*, *Roseburia inulinivorans*, *Escherichia coli*, and  
76 *Bifidobacterium breve*) can produce 1,2-propanediol from the fermentation of either  
77 rhamnose or fucose (3, 5, 17, 18). Other bacteria such as *Eubacterium hallii* and  
78 *Lactobacillus reuteri* do not utilize fucose or rhamnose directly but metabolize 1,2-  
79 propanediol (19-21) and convert it to propanol and propionate (21, 22). Cross-feeding of  
80 1,2-propanediol between *Eu. hallii* and *Bifidobacterium* species based on metabolism of  
81 mucin and HMO has been studied *in vitro* (3, 4, 6). Although cross-feeding based on  
82 1,2-propanediol is considered important in gut ecosystems (5) and has been shown to  
83 confer a fitness advantage *in vivo* for pathogens such as *Salmonella* spp. (23), its  
84 ecological importance for bacterial gut symbionts, and the underlying mechanisms by  
85 which it impacts bacterial performance in the gut, have not been empirically established  
86 *in vivo* in a tractable experimental model.

87 In both *Eu. hallii* and *L. reuteri*, 1,2-propanediol is metabolized through the  
88 cobalamin-dependent glycerol/diol dehydratase encoded in the *pduCDE* operon, found  
89 in the *pdu-cbi-cob-hem* gene (*pdu*) cluster (19-21). The glycerol/dehydratase converts  
90 1,2-propanediol to propionaldehyde and further to propanol and propionate and have  
91 been shown to increase growth rates for *L. reuteri in vitro* (19, 24). In *L. reuteri*, the *pdu*  
92 cluster is observed predominantly in two *L. reuteri* phylogenetic lineages that are  
93 dominated by strains from human, herbivore, and chicken origin, but rarer in strains  
94 from other hosts such as mice and rats (25). This distribution suggests that the cluster  
95 constitutes an adaptation to the characteristics of the gastrointestinal tract of specific  
96 hosts (26, 27). In the murine forestomach, which is densely colonized by *L. reuteri* (25),  
97 fermentable mono- and disaccharides are in ample supply (28). In contrast, distal

portions of the human intestinal tract are characterized by low concentrations of mono- and disaccharides. The *pdu* cluster may therefore constitute a colonization factor in the distal regions of the gut, to take advantage of 1,2-propanediol produced by other microbes (19, 25).

Most studies on diol-metabolism in *L. reuteri* have focused on the synthesis of the antimicrobial intermediate  $\beta$ -hydroxypropionaldehyde (reuterin) from glycerol, which is also mediated by the *pdu* operon (29). It remains unclear whether *L. reuteri* uses the *pdu* operon to engage in cross-feeding interrelationships with other gut bacterial species that produce 1,2-propanediol in its gastrointestinal habitat, and how this interaction contributes to its ecological competitiveness. It was therefore the aim of this study to characterize the ecological importance of 1,2-propanediol-cross-feeding between *L. reuteri* and the 1,2-propanediol producers *Bifidobacterium breve* and *Escherichia coli*, both *in vitro* and in the digestive tract of mice.

## Results

### 1,2-propanediol enhances growth of *L. reuteri* *in vitro*

We first sought to confirm the importance of 1,2-propanediol metabolism for *L. reuteri*. *L. reuteri* ATCC PTA 6475 (referred to as *L. reuteri* PTA 6475) growth *in vitro*. The presence of 1,2-propanediol in the medium containing glucose improved growth rates and increased the final cell density of the strain when compared to the growth solely on glucose or to growth of a *pduCDE* deletion mutant (referred to as *L. reuteri*  $\Delta$ *pduCDE*) (Fig. 1A). Neither *L. reuteri* PTA 6475 nor *L. reuteri*  $\Delta$ *pduCDE* were able to

120 use 1,2-propanediol as the sole carbon source for growth (Fig. 1A). To confirm that the  
121 enhanced growth of *L. reuteri* PTA 6475 was due to 1,2-propanediol metabolism, the  
122 metabolic end-products in the supernatant were measured using HPLC. As shown in  
123 Figure 1B and C, *L. reuteri* PTA 6475 but not *L. reuteri*  $\Delta pduCDE$  converted 1,2-  
124 propanediol to propanol and low concentrations of propionate. For *L. reuteri* PTA 6475  
125 but not *L. reuteri*  $\Delta pduCDE$ , utilization of 1,2-propanediol resulted in acetate production  
126 and decreased production of ethanol (Fig. 1D & E; Fig. S1A & B). These findings  
127 confirm that *L. reuteri* PTA 6475 is able to disproportionate 1,2-propanediol to propanol  
128 and propionate (22). However, 1,2-propanediol is not used as sole substrate but co-  
129 metabolized with glucose. Predominant production of propanol demonstrates that the  
130 reducing branch of the propanediol pathway is preferred over the oxidizing branch to  
131 regenerate electron acceptors, thus enhancing acetate formation and ATP production,  
132 and therefore growth (30).

### 133 **Interspecies cross-feeding of 1-2-propanediol enhances growth of *L. reuteri* in** 134 ***vitro***

135 We developed an experimental system to study cross-feeding between *L. reuteri*  
136 and gut bacteria that produce 1,2-propanediol. We chose *B. breve* and *E. coli* which are  
137 known to produce 1,2-propanediol from fucose and rhamnose, respectively, which are  
138 substrates not utilized by *L. reuteri*. Since growth rates and growth conditions differ  
139 among *L. reuteri*, *B. breve*, and *E. coli* are, cross-feeding was not studied in co-culture.  
140 Instead, *B. breve* and *E. coli* were first grown under their respective optimal conditions  
141 and on the specific substrates that result in the production of 1,2-propanediol. Spent  
142 supernatant obtained from these fermentations was then supplemented with glucose



143 and half-strength mMRS (conditioned media; see Materials and Methods) and used for  
144 analyzing growth kinetics and metabolite production of *L. reuteri* strains (Table 1).  
145 Conditioned media from an isogenic mutant of *B. breve* with an insertion mutation in  
146 *fucP* (encoding the L-fucose transporter) that is unable to metabolize fucose into 1,2-  
147 propanediol served as a control (Table 1).

148 *B. breve* UCC2003 and its *fucP* mutant *B. breve* UCC2003-*fucP* were grown in a  
149 medium containing cellobiose with and without fucose. Fucose does not support the  
150 growth of *B. breve* UCC2003 yet is co-utilized with cellobiose to produce 1,2-  
151 propanediol. Importantly, neither fucose or cellobiose are metabolized by *L. reuteri* (20).  
152 In the fucose/cellobiose-containing medium, *B. breve* UCC2003 and *B. breve*  
153 UCC2003-*fucP* reached similar cell density after 24 hours of growth (Fig. S2). *L. reuteri*  
154 PTA 6475 reached a significantly higher OD<sub>600</sub> and showed elevated growth rate when  
155 grown in conditioned medium with supernatant of *B. breve* UCC2003 grown with fucose  
156 and cellobiose when compared to *L. reuteri*  $\Delta pduCDE$  or *L. reuteri* PTA 6475 grown in  
157 conditioned medium of *B. breve* UCC2003 grown in the absence of fucose (Fig. 2A).  
158 The growth advantage of *L. reuteri* PTA 6475 was not observed in conditioned media  
159 from the *B. breve* UCC2003-*fucP*, even if grown in the presence of fucose (Fig. 2B).  
160 HPLC analysis confirmed the presence of 1,2-propanediol in the conditioned medium of  
161 *B. breve* UCC2003 but not *B. breve* UCC2003-*fucP* grown with fucose (Fig. S3A), and  
162 showed that enhanced growth of *L. reuteri* PTA 6475 was linked to the conversion of  
163 1,2-propanediol to propanol, which was not detected in *L. reuteri*  $\Delta pduCDE$  cultures  
164 (Fig. 2C & D). Propionate, acetate, and ethanol could not be quantified as unknown  
165 compounds in the conditioned media interfered with the metabolite analysis. Fucose

166 and cellobiose did not alter the growth kinetics of the *L. reuteri* strains when grown in  
167 glucose (Fig. S4A), and they did not serve as growth substrates on their own (Fig. S4B),  
168 confirming that the enhanced growth in *L. reuteri* PTA 6475 was not due to a direct  
169 effect of residual concentrations of these sugars in the spend supernatant.

170 1,2-propanediol results also from the fermentation of rhamnose by *E. coli*  
171 MG1655 (Fig S3B). Cross-feeding experiments revealed that *L. reuteri* PTA 6475 had a  
172 higher growth rate and reached a significantly higher cell density when grown in  
173 conditioned medium of *E. coli* grown on rhamnose when compared to *L. reuteri*  
174  $\Delta pduCDE$ , or *L. reuteri* PTA 6475 grown in conditioned medium from *E. coli* that did not  
175 contain rhamnose (Fig. 3A). Importantly, growth experiments of *L. reuteri* strains in  
176 media with rhamnose with or without glucose confirmed that rhamnose could neither be  
177 used as a carbon source nor alter growth (Fig. S4C & D). Metabolite analysis of  
178 fermentations conducted in the conditioned medium of *E. coli* grown on rhamnose  
179 revealed that *L. reuteri* PTA 6475, and not *L. reuteri*  $\Delta pduCDE$ , could metabolize 1,2-  
180 propanediol produced by *E. coli* and form propanol, propionate, and acetate (Fig. 3B-D;  
181 Fig. S5). Metabolite interference in the conditioned media did not allow the  
182 quantification of ethanol by HPLC.

183 Together, these findings demonstrate that *L. reuteri* PTA 6475 utilizes 1,2-  
184 propanediol produced by *B. breve* and *E. coli* from the fermentation of deoxyhexose  
185 sugars as an electron acceptor, enhancing its growth capabilities.

186

187 **Importance of 1,2-propanediol cross-feeding in the gastrointestinal tract**

188 The ecological relevance of cross-feeding based on 1,2-propanediol in the  
189 gastrointestinal tract was investigated with a series of colonization experiments in  
190 gnotobiotic mice (Fig 4; Table S1 & S2). Germ-free Swiss-Webster mice (6-16 weeks  
191 old; male and female) were housed in individually ventilated cages (groups of 2-3). A  
192 fat-free diet was used in order to avoid possible confounding affects from the hydrolysis  
193 of dietary triglyceride fats, which is a source of glycerol (also utilized via the *pdu* cluster  
194 and enhances growth). Fecal cell numbers of colonizing strains were determined by  
195 selective plating (see Materials and Methods).

196 As described earlier, *B. breve* produces 1,2-propanediol from fucose. Host  
197 mucins are an intrinsic source of fucose in the gastrointestinal tract, but *B. breve* does  
198 not possess glycosidases required for mucin degradation (9). 1,2-Propanediol cross-  
199 feeding between *B. breve* and *L. reuteri* was therefore studied in triple-species  
200 associated mouse in the presence of the mucinolytic strain *B. bifidum* PRL2010, which  
201 is capable of degrading mucin and releasing fucose without producing 1,2-propanediol  
202 (Fig. 4A; Table S1) (9). We first tested wild-type and mutant *L. reuteri* strains separately.  
203 Mice were gavaged with an inoculum that contained *B. bifidum* PRL2010, *B. breve*  
204 UCC2003 or its *fucP* mutant, and either *L. reuteri* PTA 6475 or *L. reuteri*  $\Delta pduCDE$   
205 (Table S1, inocula A-D). In these experiments, the two *Bifidobacterium* strains formed  
206 stable populations of  $\sim 10^8$ - $10^9$  CFU/g (Fig. S6A-H), while the *L. reuteri* strains colonized  
207 at  $\sim 10^6$ - $10^8$  CFU/g (Fig. S7A). When colonizing with wild-type *B. breve* UCC2003, *L.*  
208 *reuteri* PTA 6475 formed higher populations than *L. reuteri*  $\Delta pduCDE$  over the duration  
209 of the experiment. Contrary, fecal cell numbers of *L. reuteri* PTA6475 were  
210 consistently lower than *L. reuteri*  $\Delta pduCDE$  when colonizing with *B. breve* UCC2003-

211 fucP (but differences did not reach statistical significance due to the high variation  
212 between mice) (Fig. S7A). However, normalized ratios (see Materials and Methods)  
213 between fecal cell numbers of *L. reuteri* PTA 6475 and *L. reuteri*  $\Delta pduCDE$  were  
214 significantly higher in mice colonized with *B. breve* UCC2003 as compared to mice  
215 colonized with *B. breve* UCC2003-fucP (Fig. 5A). Interestingly, while the ratio between  
216 *L. reuteri* PTA 6475 and *L. reuteri*  $\Delta pduCDE$  was greater than 1 when the *L. reuteri*  
217 strains were co-colonized with *B. breve* UCC2003, it was substantially lower than 1  
218 when co-colonized with *B. breve* UCC2003-fucP (Fig. 5A). These finding suggests that  
219 the *pdu* cluster confers a fitness burden on *L. reuteri* unless 1,2-propanediol is provided,  
220 under which the cluster becomes advantageous.

221 In a parallel set of experiments, we tested the importance of 1,2-propanediol  
222 cross-feeding in gnotobiotic mice that were gavaged with *B. bifidum* PRL2010, either *B.*  
223 *breve* UCC2003 or *B. breve* UCC2003-fucP, and both *L. reuteri* PTA 6475 and *L. reuteri*  
224  $\Delta pduCDE$  (wild-type and mutant) in direct competition (Table S1, inocula E & F). In  
225 agreement with the experiments with only one *L. reuteri* strain, *Bifidobacterium* species  
226 formed stable populations that were comparable among groups ( $\sim 10^8$ - $10^9$  CFU/g; Fig.  
227 S6I-L). *L. reuteri* formed stable populations ( $\sim 10^6$ - $10^8$  CFU/g), and concordant with the  
228 experiments using single strains of *L. reuteri*, cell counts of *L. reuteri* PTA 6475 were  
229 higher than the mutant in mice colonized with *B. breve* UCC2003 as compared to the  
230 *B. breve* UCC2003-fucP (but differences were not statistically significant) (Fig. S7B).  
231 However, significant differences were observed between the relative proportions of the  
232 two *L. reuteri* strains. Specifically, *L. reuteri* PTA 6475 reached significantly higher  
233 proportions (>75 %) in mice colonized with *B. breve* UCC2003 as compared to *B. breve*

234 UCC2003-fucP (Fig. 5B). Interestingly, *L. reuteri*  $\Delta pduCDE$  reached ~75 % of the total  
235 *Lactobacillus* population in mice colonized with *B. breve* UCC2003-fucP (Fig. 5B),  
236 supporting the notion that the *pdu* cluster is a fitness burden to *L. reuteri* unless 1,2-  
237 propanediol is provided. Overall, these observations demonstrated that *B. breve*  
238 UCC2003 can provide 1,2-propanediol as the result of a trophic chain from the  
239 degradation of mucin by *B. bifidum* PRL2010 that facilitates colonization of *L. reuteri* in  
240 the gastrointestinal tract (9).

241 A set of dual-associated gnotobiotic mouse experiments were also conducted to  
242 test if the production of 1,2-propanediol, from the metabolism of rhamnose by *E. coli*,  
243 influences the fitness of *L. reuteri* in the gastrointestinal tract. Mice were colonized  
244 either with *E. coli* and *L. reuteri* PTA 6475 and *L. reuteri*  $\Delta pduCDE$  alone or in  
245 competition. Rhamnose was provided to mice in the drinking water (Fig. 4B; Table S2).  
246 Stable populations of *E. coli* were reached in all mice ( $\sim 10^9$  CFU/g), and the provision of  
247 rhamnose led to an ~10-fold increase in fecal cell numbers of *E. coli* (Fig. S8). Contrary  
248 to the findings with gnotobiotic mice colonized by bifidobacteria, *L. reuteri*  $\Delta pduCDE$   
249 colonized with higher cell numbers and outcompeted *L. reuteri* PTA 6475 under all  
250 conditions tested (Fig. 5C & D). This was indicated by ratios of less than 1 between *L.*  
251 *reuteri* PTA 6475 and *L. reuteri*  $\Delta pduCDE$  in mice colonized with single *L. reuteri* strains  
252 (Fig. 5C) and an enrichment of the mutant to ~60 % of the total *Lactobacillus* population  
253 in competition experiments (Fig. 5D). The latter results resample those obtained with  
254 the triple-species *Bifidobacterium* experiments in mice colonized with *B. breve*  
255 UCC2003-fucP (Fig. 5), confirming the fitness burden of the *pdu* cluster.

256

257 **Discussion**

258 Cross-feeding between members of the gut microbiota based on 1,2-propanediol is  
259 considered an important ecological process that shapes the gut microbiome and its  
260 metabolism. However, conclusions have so far been derived from in vitro experiments  
261 and metagenomic predictions. In this study, we demonstrate that *L. reuteri* engages in  
262 trophic interactions with bacteria that are common in the human gut and provide 1,2-  
263 propanediol, which is used predominantly to regenerate reduced metabolic cofactors.  
264 Using isogenic mutants in both the bacterium that produces 1,2-propanediol and *L.*  
265 *reuteri*, we ensured that this cross-feeding interaction is in fact based on this metabolic  
266 intermediate. We further established that the *pduCDE* genes constitute a fitness burden  
267 for *L. reuteri* in the gut unless 1,2-propanediol is provided, which makes the cluster  
268 ecologically advantageous. Our findings therefore provide insight into both the  
269 ecological role of the *pdu* cluster in *L. reuteri* and how it evolved.

270 Our results demonstrate that *in vitro*, *L. reuteri* can obtain a growth advantage by  
271 cross-feeding from 1,2-propanediol derived from the fermentation of fucose and  
272 rhamnose by *B. breve* and *E. coli*, respectively. These findings extend previous work  
273 showing that *pdu* cluster-containing *L. reuteri* strains grow at a faster rate and to a  
274 higher cell yield in the presence of glycerol or 1,2-propanediol (19). Contrary to findings  
275 with *L. reuteri* DSM 20016, which produces propanol and propionate in equimolar  
276 concentrations through disproportionation when utilizing 1,2-propanediol as a sole  
277 substrate (22), *L. reuteri* PTA 6475 produces propanol in excess over propionate  
278 (Figure 1B) and enhances acetate formation (Fig. 1D) when utilizing 1,2-propanediol  
279 together with glucose. These metabolic patterns suggest that when *L. reuteri* PTA 6475

grows with glucose, 1,2-propanediol functions mainly as an electron acceptor, allowing the conversion of acetyl-phosphate to acetate and to generate an extra ATP (30). Apart from enhancing bacterial growth, 1,2-propanediol metabolism by *L. reuteri* can therefore contribute to SCFA formation in the gut, which might have health implications as propionate and acetate impact host physiology, e.g. by contributing to gluconeogenesis in the liver, reducing cholesterol, and promoting satiety (5, 18).

Our experiments in gnotobiotic mice provide empirical evidence for the ecological relevance of 1,2-propanediol cross-feeding for the ecological performance of a gut symbiont in the gastrointestinal tract. The use of the respective *pduCDE* and *fucP* mutants of *L. reuteri* and *B. breve* in the mouse experiments indicated that this trophic interaction was specifically based on 1,2-propanediol produced through the fermentation of fucose. Since *B. breve* cannot degrade mucins, and the mouse feed was devoid of fucose, the findings suggests that *L. reuteri* benefits from 1,2-propanediol resulting from interspecies trophic interactions between bifidobacteria, in which *B. bifidum* PRL2010 degrades host mucin and provides fucose for *B. breve* to produce 1,2-propanediol (9). The hydrolysis of mucin is suggested to play a key role in the facilitation of bacterial species in the gut microbiota and have been demonstrated *in vitro* (7, 31, 32) and with bacterial pathogens *in vivo* (e.g. *Salmonella* spp. and *Clostridium difficile*) (23, 33). Our data suggests that it also plays a role in cross-feeding interactions among symbionts or commensals, ultimately conferring a fitness advantage to *L. reuteri* in the murine gut through the provision of 1,2-propanediol.

Although our experiments demonstrated 1,2-propanediol cross-feeding between bifidobacteria and *L. reuteri*, equivalent findings were not observed in mouse



303 experiments with *L. reuteri* and *E. coli*. This observation can potentially be attributed to  
304 a phenomenon called carbon catabolite repression or the “all-or-none” effect in *E. coli*,  
305 in which a hierarchy-based regulatory system controls the sequential uptake of carbon  
306 sources (34). The mouse diet was highly saturated with glucose (34.4 % w/w) and, *in*  
307 *vitro*, the presence of glucose suppresses the uptake of other carbohydrates in *E. coli*  
308 (35), which is in agreement with our finding that *E. coli* MG1655 did not produce 1,2-  
309 propanediol from rhamnose while in the presence of glucose (Fig. S3B). Hence, it is  
310 possible that the uptake and metabolism of rhamnose into 1,2-propanediol by *E. coli*  
311 was suppressed in the murine gut.

312 Interestingly, the mouse experiments consistently revealed a fitness burden of  
313 the *pduCDE* genes when 1,2-propanediol was absent. Fitness trade-offs are well  
314 understood in the evolution of antibiotic resistance in bacteria, where resistance genes  
315 are costly and lead to a reduction of growth (36), and are therefore often lost in the  
316 absence of antibiotic pressure (37). Our findings indicate that genes that facilitate cross-  
317 feeding interactions are also subjected to fitness trade-offs in that they are only  
318 beneficial when the metabolite is provided. Such trade-offs have also been shown in  
319 cross-feeding based on the exchange of carbohydrates. *Bacteroides ovatus* possesses  
320 an enzyme system dedicated to the digestion of polysaccharides that does not directly  
321 benefit itself, but rather cooperative members of the gut microbiota through reciprocal  
322 cross-feeding. This enzyme system is energetically unfavorable and in the absence of a  
323 reciprocating species, a knock-out mutant strain can outcompete the enzyme-encoding  
324 wild-type (38). In *L. reuteri*, the fitness burden of the *pdu* genes provides a possible  
325 explanation for the evolution of the *pdu* cluster (26). Although likely ancestral to



326 currently known lineages of *L. reuteri*, there is evidence of a deletion of the *pdu* cluster  
327 from most rodent strains (25, 26). Growth substrates are abundant in the forestomach  
328 and bacteria providing 1,2-propanediol are absent (25, 26), which might have led to loss  
329 of the *pdu* cluster as it was evolutionary advantageous, in agreement with the Black  
330 Queen Hypothesis (39). In the human distal gut, 1,2-propanediol is likely provided  
331 through other microbiota members, which may explain why the *pdu* cluster is conserved  
332 among these strains (26, 27).

333         Although cross-feeding based on 1,2-propanediol is only one of many trophic  
334 interactions that establish interactive networks within the gut microbiota, this study  
335 provides important information as it establishes the importance and consequences of  
336 cross-feeding for the ecological performance of the involved members. Such knowledge  
337 has repercussions for our understanding on the ecological and evolutionary forces that  
338 shape gut ecosystems and determine how they function (38, 40-42). In addition, an  
339 understanding of mutualistic interactions has important implications as it can be  
340 translated into improved microbial-based strategies to modulate gut microbiomes (i.e.  
341 probiotics). A challenge encountered in the field of probiotics is that gut ecosystems are  
342 homeostatic, resilient to change, and thus difficult to modulate, and most probiotics do  
343 not persist or change the resident community (43-46). One solution to this problem is  
344 the adoption of an ecological framework for probiotic applications (44). A consideration  
345 of the mutualistic and facilitative interactions between community members can be used  
346 for designing probiotic strain mixtures or personalized probiotic applications with the  
347 goal to achieve a more successful long-term persistence, which might be beneficial for  
348 certain applications. For example, 1,2-propanediol cross-feeding could be considered in

349 generating probiotic products by pairing *L. reuteri* with *Bifidobacterium* species that  
350 release fucose from the degradation of host-derived substrates and convert it into 1,2-  
351 propanediol (9, 12). Additionally, bifidobacteria are more prevalent in the infant gut (47,  
352 48) and some strains only partly utilize HMOs, releasing fucose, possibly forming an  
353 effective synergistic combination with *L. reuteri* (3, 32, 40). Furthermore, cross-feeding  
354 of 1,2-propanediol derived from gut symbionts and *S. enterica* serovar Typhimurium has  
355 been demonstrated to play a role in promoting pathogen expansion in the gut (23). *L.*  
356 *reuteri* could play a therapeutic role in excluding pathogenic *Salmonella* during  
357 gastroenteritis, by directly competing for the intermediary metabolite. Overall, this  
358 information could not only be used to formulate probiotic mixtures and synbiotic  
359 products, but potentially personalize probiotic applications based on the baseline  
360 microbiome (43).

361

## 362 **Materials and Methods**

### 363 **Bacterial strains and culture conditions**

364 Bacterial strains used in this study are listed in Table 2. *L. reuteri* strains were grown  
365 in de Man, Rogosa, and Sharpe (MRS) medium (Difco) in an anaerobic chamber (gas  
366 mix of 5 % CO<sub>2</sub>, 5 % H<sub>2</sub>, and 90 % N<sub>2</sub>). *B. bifidum* PRL2010, *B. breve* UCC2003, and *B.*  
367 *breve* UCC2003-fucP were grown anaerobically in MRS medium supplemented with  
368 0.05 % L-cysteine. *E. coli* were grown in Luria-Bertani (LB) broth with agitation. All  
369 incubations were performed at 37 °C.

### 370 **Evaluation of the impact of 1,2-propanediol on *L. reuteri* ATCC PTA 6475 growth**

371 Overnight cultures of *L. reuteri* PTA 6475 and *L. reuteri*  $\Delta pduCDE$  were inoculated at  
372 1 % into 15 ml of half-strength mMRS (49) containing 25mM glucose alone, 50 mM of  
373 1,2-propanediol (Sigma-Aldrich) alone, or 25mM glucose plus 50mM of 1,2-propanediol.  
374 Growth of cell cultures were monitored based on OD<sub>600</sub> with a spectrophotometer every  
375 3 h over the span of 12 h. One mL samples were collected for HPLC analysis every 3 h.  
376 All experiments were performed in triplicate at 37 °C under anaerobic conditions.

#### 377 ***In vitro* production of 1,2-propanediol and cross-feeding assay development**

378 Pre-cultures of *B. breve* strains and *E. coli* were prepared as follows. Full-strength  
379 mMRS supplemented with 30 mM cellobiose  $\pm$  30 mM L-fucose were inoculated with 1  
380 % of overnight cultures of *B. breve* UCC2003 or *B. breve* UCC2003-fucP. Full-strength  
381 mMRS containing 25 mM of glucose or 30 mM of L-rhamnose were inoculated with 1 %  
382 of overnight *E. coli* MG1655 cultures. These fermentations were conducted under  
383 anaerobic conditions for 24 h at 37 °C. Conditioned media were prepared from pre-  
384 cultures as follows. Cells were removed from pre-cultures by centrifugation (5000 x g for  
385 10 minutes) after which the supernatant was collected. The supernatant was  
386 supplemented with half-strength mMRS from scratch (50 % w/v; mMRS dry reagents)  
387 and glucose (25 mM; dry reagent) to the supernatant. These were further adjusted to  
388 pH 6.6 and filter sterilized (0.22  $\mu$ m), stored at 4 °C and used within 48 h. Conditioned  
389 media are described in Table 1. For growth experiments, conditioned media were  
390 inoculated with *L. reuteri* strains (1 % inoculation). Growth was monitored for 12 h by  
391 measuring OD<sub>600</sub> with a spectrophotometer and 1 mL samples for HPLC analysis were  
392 collected every 3 h. All experiments were performed in triplicate under anaerobic  
393 conditions at 37 °C.

394 **Experiments in gnotobiotic mice**

395 All animal experiments were performed with the approval of the Animal Care and  
396 Use Committee (ACUC) of the University of Alberta (AUP 00002764). Germ-free Swiss-  
397 Webster mice (6-16 weeks of age, male and female) were bred and maintained in the  
398 Health Sciences Laboratory Animals Services (HSLAS) Facility at the University of  
399 Alberta. Mice were randomly selected and moved from a flexible-film isolator and  
400 housed in sterile, individually ventilated, positive-pressured biocontainment cages for  
401 the duration of the experiments (IsoCage P Biocontainment; Tecniplast). To avoid  
402 possible confounding effects of glycerol, which is also utilized by the *pdu* cluster-  
403 encoded diol/glycerol dehydratase (50) , an irradiated fat-free diet (34.4 % glucose and  
404 34.4 % cornstarch; Teklad TD.180765) was used in order to minimize possible  
405 interference from the hydrolysis of triglyceride fats. After transfer to the biocontainment  
406 cages, mice were fed with the new diet for 3 d before colonization with the bacteria.

407 To study the cross-feeding of 1,2-propanediol in the gastrointestinal tract, groups of  
408 mice ( $n=5$ ), 2 to 3 mice per cage, were assigned to receive either *Bifidobacterium-L.*  
409 *reuteri* triple-species mixtures (Table S1) or *E. coli-L. reuteri* double-species mixtures  
410 (Table S2). To test for cross-feeding of 1,2-propanediol produced from mucin derived  
411 fucose, mice were gavaged with *Bifidobacterium-L. reuteri* triple-species mixtures  
412 containing *B. bifidum* PRL2010, either *B. breve* UCC2003 or *B. breve* UCC2003-fucP,  
413 and either *L. reuteri* PTA 6475 or *L. reuteri*  $\Delta pduCDE$  (single *L. reuteri* strains), or both  
414 strains in competition (Table S1). To test for cross-feeding of dietary rhamnose, we  
415 gavaged mice with *E. coli-L. reuteri* double-species mixtures containing *E. coli* and *L.*  
416 *reuteri* PTA 6475 or *L. reuteri*  $\Delta pduCDE$  (single *L. reuteri* strains), or both strains in

417 competition (Table S2). Rhamnose was provided in the drinking water (2 % w/v). Each  
418 mouse was gavaged with 200  $\mu$ L of the corresponding bacterial cell mixtures containing  
419  $\sim 10^8$  viable cells of each strain. Fecal pellets were collected from individual mice 1, 3, 5,  
420 and 7 days after inoculation and plated. Selective plating was used to enumerate  
421 bacterial cells in fecal samples as follows: Modified Rogosa agar plates were used to  
422 quantify *L. reuteri* strains (51). *L. reuteri* PTA 6475 and *L. reuteri*  $\Delta$ pduCDE were  
423 differentiated using a reuterin hydrazone detection assay (52). MacConkey agar was  
424 used for quantifying *E. coli*. *Bifidobacterium* were selected using *Bifidobacterium*  
425 selective iodoacetate mupirocin (BSIM) agar as previously described (48). *B. bifidum*  
426 PRL2010 and *B. breve* strains were differentiated based on colony morphology.

#### 427 **Metabolite analysis of post-fermentation**

428 1,2-Propanediol, propanol, propionate, acetate, and ethanol were measured using  
429 HPLC. A BioRad Aminex HPX-87H column (300 mm x 7.8 mm) and a refractive index  
430 detector was used (HPLC-RI). Samples taken from fermentations were mixed with 70 %  
431 HClO<sub>4</sub> (0.005 % v/v), stored at 4 °C overnight to precipitate proteins, centrifuged (18,800  
432 x g for 5 minutes), filtered (0.22  $\mu$ m), and stored at -20 °C before injection into HPLC.  
433 Ten  $\mu$ L were injected and eluted with 5 mM H<sub>2</sub>SO<sub>4</sub> at a flow rate of 0.4 ml/min at 70 °C.  
434 1,2-Propanediol, propanol, propionate, acetate, and ethanol were quantified using  
435 external standards.

#### 436 **Statistical analysis**

437 Statistical significance between *L. reuteri* growth curves were determined by two-  
438 way analysis of variance (ANOVA) with Bonferroni multiple comparisons test ( $\alpha = 0.05$ ).

439 An unpaired two-tailed Student's *t*-test was used to analyze significance between *B.*  
440 *breve* growth in mMRS supplemented with cellobiose ± fucose.

441 Comparisons between *L. reuteri* PTA 6475 and *L. reuteri*  $\Delta pduCDE$  CFU recovered  
442 from fecal samples over the duration of the gnotobiotic mice experiments were  
443 performed by unpaired two-tailed Student's *t*-test. Tests were conducted between *L.*  
444 *reuteri* strains that were associated with (i) *B. breve* UCC2003 or (ii) *B. breve* UCC2003-  
445 fucPin the triple-species experiments and *E. coli*-*L. reuteri* double-species experiments  
446 with rhamnose (iii) present or (iv) absent.

447 For the gnotobiotic mice experiments inoculated with the 'single *L. reuteri* strain'  
448 mixtures (Table S1 & 2), CFU of *L. reuteri* PTA 6475 and *L. reuteri*  $\Delta pduCDE$  recovered  
449 from mouse feces were used to produce normalized ratios. Ratios were generated  
450 using the formula (equation 1) below, where  $a_n$  is a CFU value for *L. reuteri* PTA 6475 -  
451 from a single mouse - used in the comparison,  $b_n$  is the CFU value of *L. reuteri*  
452  $\Delta pduCDE$  from each mouse in the group, and  $n_b$  is the total population of mice  
453 inoculated with the mutant strain used in the experiment.

$$Normalized\ ratio = \frac{a_n}{\sum \left( \frac{(b_1 + b_2 + \dots + b_n)}{n_b} \right)} \quad (1)$$

454  
455 The formula was used to generate sets of ratios for the following comparisons from  
456 the 'Single *L. reuteri* strain' mice experiments: (i) *B. breve* UCC2003 vs *B. breve*  
457 UCC2003-fucP and (ii) *E. coli*-*L. reuteri* double-associated mice in the presence vs the

458 absence of rhamnose. Statistical significance between the sets of ratios were  
459 determined by Mann-Whitney U test ( $P$  value  $< 0.05$ ).

460 Fisher's exact test was used to determine statistical significance between *L. reuteri*  
461 population frequencies from murine groups inoculated with '*L. reuteri* strains in  
462 competition' mixtures ( $P$  value  $< 0.05$ ). This was performed between groups of mice  
463 from either: (i) *Bifidobacterium-L. reuteri* triple-species associations including *B. breve*  
464 UCC2003 vs *B. breve* UCC2003-fucP(ii) *E. coli-L. reuteri* double-species associations  
465 with murine diet supplemented with rhamnose vs without rhamnose. Statistical analyses  
466 were performed using GraphPad Prism 6.07.

467

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474 the *B. bifidum* PRL2010 strain.

475

#### 476 **Author contributions**

477 C.C.C. designed and conducted the experiments, collected and analyzed data,  
478 and wrote the manuscript. R.M.D. contributed to conceiving the project, the

479 development of experimental approaches, supervision, intellectual leadership, and  
480 wrote the manuscript. X.B.L contributed to experimental designs and edited the  
481 manuscript. M.E.P.M contributed to the design of animal experiments, and contributed  
482 to the manuscript through editing and generation of figures. S.T. was responsible for  
483 animal husbandry and oversaw animal experiments. J.H.O. and J.P.V. generated the  
484 mutant *L. reuteri* ATCC PTA 6475  $\Delta pduCDE$  strains and contributed to manuscript  
485 editing. F.L. contributed to supervision and manuscript editing. D.V. provided the  
486 *Bifidobacterium bifidum* PRL2010, *Bifidobacterium breve* UCC2003, *Bifidobacterium*  
487 *breve* UCC2003-fucP strains, contributed to conceiving animal experiments, and gave  
488 technical and conceptual advice. M.G.G. oversaw analytical analysis and data  
489 interpretation, and contributed to supervision and manuscript editing. J.W. conceived  
490 project, contributed to the conceptualization of the experiments, supervised data  
491 analysis and interpretation, and wrote the manuscript.

492

493



494 Table 1. Media used for *in vitro* cross-feeding experiments

Fermenting Strain	Deoxyhexose Sugar	Other carbohydrates	Abbreviation	Purpose
<i>B. breve</i> UCC2003	N/a	30 mM Cellobiose	BM (C)	Control for <i>L. reuteri</i> growth absent of 1,2-propanediol production
	30 mM Fucose	30 mM Cellobiose	BM (CF)	For the study of effect of 1,2-propanediol produced from Fucose fermentation on <i>L. reuteri</i>
<i>B. breve</i> UCC2003-fucP	N/a	30 mM Cellobiose	B-fucP M (C)	Control for <i>L. reuteri</i> growth absent of 1,2-propanediol production
	30 mM Fucose	30 mM Cellobiose	B-fucP M (CF)	Control for <i>L. reuteri</i> growth absent of 1,2-propanediol production
<i>E. coli</i> MG1655	N/a	25 mM Glucose	EM (G)	Control for <i>L. reuteri</i> growth absent of 1,2-propanediol production
	30 mM Rhamnose	N/a	EM (R)	For the study of effect of 1,2-propanediol produced from Rhamnose fermentation on <i>L. reuteri</i>

495 N/a: not applicable

496

497

498

499 Table 2. Strains used in this study

Species (strain code)	Origin	Relevant features	Reference
<i>Lactobacillus reuteri</i> ATCC PTA 6475	Breast Milk	1,2-propanediol utilizer with complete <i>pdu</i> cluster	BioGaia; (53)
<i>L. reuteri</i> $\Delta$ <i>pduCDE</i>	Isogenic mutant of PTA 6475	Deletion mutant of Glycerol/diol dehydratase genes ( <i>pduCDE</i> )	(53)
<i>Escherichia coli</i> MG1655	Lab-derived strain	L-rhamnose utilizer, 1,2-propanediol producer	The Coli Genetic Stock Center (CGSC)
<i>Bifidobacterium bifidum</i> PRL2010	Infant stool	Mucin degrader, L-fucose producer	(32)
<i>Bifidobacterium breve</i> UCC2003	Infant stool	L-fucose utilizer, 1,2-propanediol producer	(54)
<i>Bifidobacterium breve</i> UCC2003- <i>fucP</i>	Isogenic mutant of UCC2003	Insertion mutant of the L-fucose transporter gene ( <i>fucP</i> )	(9)

500

501

502 **Figures legends**

503 **Figure 1.** Impact of 1,2-propanediol on growth and metabolism of *L. reuteri* PTA 6475  
504 and *L. reuteri*  $\Delta pduCDE$ . (A) *L. reuteri* strains were grown in half-strength mMRS  
505 supplemented with either glucose (Glc; 25 mM), 1,2-propanediol (50 mM), or a mixture  
506 of the two. Asterisks indicate a significant difference (Two-way ANOVA;  $p < 0.001$ ) in  
507 growth of *L. reuteri* PTA 6475 on glucose plus 1,2-propanediol compared to the other  
508 conditions. (B-C) Utilization of 1,2-propanediol and production of propanol and  
509 propionate by (B) *L. reuteri* PTA 6475 and (C) *L. reuteri*  $\Delta pduCDE$  during growth on  
510 glucose in the presence of 1,2-propanediol. (D-E) Production of (D) acetate and (E)  
511 ethanol by the two strains during growth on glucose in the presence of 1,2-propanediol.

512  
513 **Figure 2.** Growth and metabolites of *L. reuteri* PTA 6475 and *L. reuteri*  $\Delta pduCDE$  in  
514 conditioned media of *B. breve* UCC2003 and *B. breve* UCC2003-fucP grown with  
515 cellobiose alone or with the addition of fucose. Growth curves of (A) *L. reuteri* strains in  
516 *B. breve* UCC2003 conditioned media and (B) *L. reuteri* in *B. breve* UCC2003-fucP  
517 conditioned media. Asterisks indicate a significant difference (Two-way ANOVA;  $p <$   
518 0.001) in growth of *L. reuteri* PTA 6475 grown in *B. breve* UCC2003 conditioned  
519 medium that had fermented cellobiose and fucose together compared to growth of *L.*  
520 *reuteri* PTA 6475 in *B. breve* UCC2003 conditioned medium without fucose or to *L.*  
521 *reuteri*  $\Delta pduCDE$  grown in all conditioned media derived from *B. breve* UCC2003. (C-D)  
522 Utilization of *B. breve* derived 1,2-propanediol present in the conditioned media and  
523 production of propanol in cultures of (C) *L. reuteri* PTA 6475 and (D) *L. reuteri*  
524  $\Delta pduCDE$  grown in the conditioned medium of *B. breve* UCC2003 grown in the

525 presence of fucose. Propionate, acetate, and ethanol concentrations could not be  
526 determined due to interference of unknown compounds in the medium. Abbreviations:  
527 BM, *B. breve* UCC2003 conditioned media; B-fucP M, *B. breve* UCC2003-fucP  
528 conditioned media; (C), pre-culture fermentations of cellobiose only; (CF), pre-culture  
529 fermentations of cellobiose with added fucose (See Table 1 for more details about  
530 media used in the study).

531

532 **Figure 3.** Growth curves and metabolites of *L. reuteri* PTA 6475 and *L. reuteri*  
533  $\Delta pduCDE$  in conditioned media of *E. coli* grown with glucose or rhamnose. (A) Growth  
534 of *L. reuteri* strains. Asterisks indicate a significant difference (Two-way ANOVA;  $p <$   
535 0.01) in growth of *L. reuteri* PTA 6475 grown in *E. coli* conditioned medium that had  
536 fermented rhamnose compared to *E. coli* conditioned medium that fermented glucose  
537 and to growth of *L. reuteri*  $\Delta pduCDE$  in both *E. coli* conditioned media fermentations of  
538 either glucose or rhamnose. (B-C) Utilization of *E. coli*-derived 1,2-propanediol, and  
539 production of propanol and propionate by (B) *L. reuteri* PTA 6475 and (C) *L. reuteri*  
540  $\Delta pduCDE$  grown in conditioned medium from *E. coli* grown with rhamnose. (D)  
541 Comparison of acetate production by the two strains grown in conditioned medium from  
542 *E. coli* grown with rhamnose. Ethanol concentrations could not be determined due to  
543 interference of an unknown compound in the medium. Abbreviations: EM, *E. coli*  
544 conditioned media; (G), pre-culture fermentation of glucose only by *E. coli*; (R), pre-  
545 culture fermentation of rhamnose only by *E. coli*. (See Table 1 for more details about  
546 media used in the study).

547

548 **Figure 4.** Graphical illustration of hypothesized trophic interactions of 1,2-propanediol  
549 in gnotobiotic mice. (A) In triple-species associated gnotobiotic mice (colonized by *B.*  
550 *bifidum*, *B. breve*, and *L. reuteri*), *B. bifidum* liberates fucose from the degradation of  
551 host mucin, which is metabolized by *B. breve* UCC2003 producing 1,2-propanediol, that  
552 is subsequently utilized by *L. reuteri* PTA 6475. (B) In dual-species (*E. coli* and *L.*  
553 *reuteri*) associated mice whose diet has been supplemented with rhamnose added  
554 through the drinking water, *E. coli* metabolizes rhamnose producing 1,2-propanediol  
555 that is subsequently utilized by *L. reuteri* PTA 6475.

556  
557 **Figure 5.** Populations of *L. reuteri* PTA 6475 and *L. reuteri*  $\Delta pduCDE$  in the  
558 gastrointestinal tract of triple-species and double-species associated gnotobiotic mice.  
559 (A) Normalized ratios between *L. reuteri* PTA 6475 to *L. reuteri*  $\Delta pduCDE$  obtained from  
560 *Bifidobacterium*-*L. reuteri* triple-species associated gnotobiotic mice in which  
561 colonization by *L. reuteri* PTA 6475 and *L. reuteri*  $\Delta pduCDE$  was tested separately. “+”  
562 indicates mice colonized with *B. breve* UCC2003 and “-” indicates mice colonized with  
563 *B. breve* UCC2003-fucP. (B) Percent CFU for *L. reuteri* PTA 6475 and *L. reuteri*  
564  $\Delta pduCDE$  as measured in triple-species associated gnotobiotic mice in which the two *L.*  
565 *reuteri* strains were tested in competition. (C) Normalized ratios between *L. reuteri* PTA  
566 6475 and *L. reuteri*  $\Delta pduCDE$  in *E. coli*-*L. reuteri* double-species associated gnotobiotic  
567 mice in which colonization of *L. reuteri* PTA 6475 and *L. reuteri*  $\Delta pduCDE$  was tested  
568 separately. (D) Percent CFU for *L. reuteri* PTA 6475 and *L. reuteri*  $\Delta pduCDE$  mutant in  
569 double-species associated gnotobiotic mice in which the two *L. reuteri* strains were  
570 tested in competition. “+” indicates the presence of rhamnose (Rha) in the diet, while “-”

571 indicates absence of rhamnose in the diet. Statistical significance for ratios and percent  
572 abundance (% CFU) was determined using Mann-Whitney test and Fisher's exact test,  
573 respectively.

574

575 **Table S1.** Strains used in experiments with *Bifidobacterium-L. reuteri* triple-species  
576 associated gnotobiotic mice.

577

578 **Table S2.** Strains used in experiments with *E. coli-L. reuteri* double-species associated  
579 mice.

580

581 **Figure S1.** Concentrations of (A) acetate and (B) ethanol in media after growth of *L.*  
582 *reuteri* PTA 6475 and *L. reuteri*  $\Delta pduCDE$  on glucose (Glc) in the presence or absence  
583 of 1,2-propanediol.

584

585 **Figure S2.** Growth of *B. breve* UCC2003 and *B. breve* UCC2003-fucP in mMRS  
586 supplemented with cellobiose (30 mM; Cell), fucose (30 mM; Fuc), cellobiose and  
587 fucose, or with no carbon source (No Carb) after 24 hours.

588

589 **Figure S3.** Total 1,2-propanediol concentrations in media after growth with (A) *B. breve*  
590 UCC2003 and *B. breve* UCC2003-fucP after fermentation of cellobiose (Cell) and  
591 cellobiose plus fucose (Cell + Fuc). (B) 1,2-propanediol concentrations in *E. coli* cultures  
592 after growth with glucose (Glc), rhamnose (Rha), or glucose and rhamnose (Glc + Rha).

593

594 **Figure S4.** Growth of *L. reuteri* PTA 6475 and *L. reuteri*  $\Delta pduCDE$  in the presence of  
595 deoxyhexose sugars. *L. reuteri* strains do not utilize (A & B) cellobiose (Cell), fucose  
596 (Fuc), or (C & D) rhamnose (Rha) as growth substrates or electron acceptors when  
597 cultured with glucose (Glc).

598

599 **Figure S5.** Acetate production of *L. reuteri* PTA 6475 and *L. reuteri*  $\Delta pduCDE$  in the  
600 conditioned media of *E. coli* fermentation of glucose and rhamnose. In the symbol  
601 labels, *E. coli* conditioned media is abbreviated as EM, followed by fermentation of  
602 glucose by *E. coli* as indicated with (G), and rhamnose with (R) (See Table 1 for more  
603 details about media used in the study).

604

605 **Figure S6.** Quantification of *Bifidobacterium* strains in fecal samples from triple-species  
606 associated gnotobiotic mice. Fecal CFU of *B. bifidum* PRL2010 recovered from of  
607 gnotobiotic mice inoculated with (A) *B. breve* UCC2003 and *L. reuteri* PTA 6475 or (B)  
608 *B. breve* UCC2003 and *L. reuteri*  $\Delta pduCDE$ . Fecal CFU of *B. bifidum* PRL2010 from  
609 gnotobiotic mice inoculated with (C) *B. breve* UCC2003-fucP and *L. reuteri* PTA 6475 or  
610 (D) *B. breve* UCC2003-fucP and *L. reuteri*  $\Delta pduCDE$ . Fecal CFU of *B. breve* UCC2003  
611 from gnotobiotic mice inoculated with (E) *B. bifidum* PRL2010 and *L. reuteri* PTA 6475  
612 or (F) *B. bifidum* PRL2010 and *L. reuteri*  $\Delta pduCDE$ . Fecal CFU of *B. breve* UCC2003-  
613 fucP from gnotobiotic mice inoculated with (G) *B. bifidum* PRL2010 and *L. reuteri* PTA  
614 6475 or (H) *B. bifidum* PRL2010 and *L. reuteri*  $\Delta pduCDE$ . Fecal CFU of *B.*  
615 *bifidum* PRL2010 from gnotobiotic mice inoculated with *L. reuteri* PTA 6475 and *L.*

616 *reuteri*  $\Delta pduCDE$  in competition as well as with (I) *B. breve* UCC2003 or (J) *B.*  
617 *breve* UCC2003-fucP. (K) Fecal CFU of *B. breve* UCC2003 from gnotobiotic mice  
618 inoculated with *B. bifidum* PRL2010 and *L. reuteri* PTA 6475 and *L. reuteri*  $\Delta pduCDE$  in  
619 competition. (L) Fecal CFU of *B. breve* UCC2003-fucP from gnotobiotic mice inoculated  
620 with *B. bifidum* PRL2010 and *L. reuteri* PTA 6475 and *L. reuteri*  $\Delta pduCDE$  in  
621 competition.

622 **Figure S7.** Quantification of *L. reuteri* strains from triple-species and double-species  
623 associated gnotobiotic mice experiments. (A-B) CFU of *L. reuteri* recovered from feces  
624 of the *Bifidobacterium* triple-species gnotobiotic mice experiments containing either (A)  
625 single *L. reuteri* strains or (B) *L. reuteri* strains in competition. “+” indicates mice  
626 colonized with *B. breve* UCC2003 and “-” indicates mice colonized with the *fucP* mutant  
627 of *B. breve* UCC2003. (C-D) CFU of *L. reuteri* recovered from feces of *E. coli* double-  
628 species gnotobiotic mice experiments from either (C) single *L. reuteri* strains or (D) *L.*  
629 *reuteri* strains in competition. “+” indicates the presence of rhamnose (Rha) in the diet,  
630 while “-” indicates absence of rhamnose in the diet.

631

632 **Figure S8.** Quantification of *E. coli* from double-species associated gnotobiotic mice  
633 experiments. CFU of *E. coli* recovered from ‘single *L. reuteri* strain’ bacterial mixtures in  
634 the (A) presence of rhamnose in the diet, (B) absence of rhamnose in the diet, and from  
635 (C) *L. reuteri* in competition where rhamnose was either supplemented into the mouse  
636 diet or not.

637



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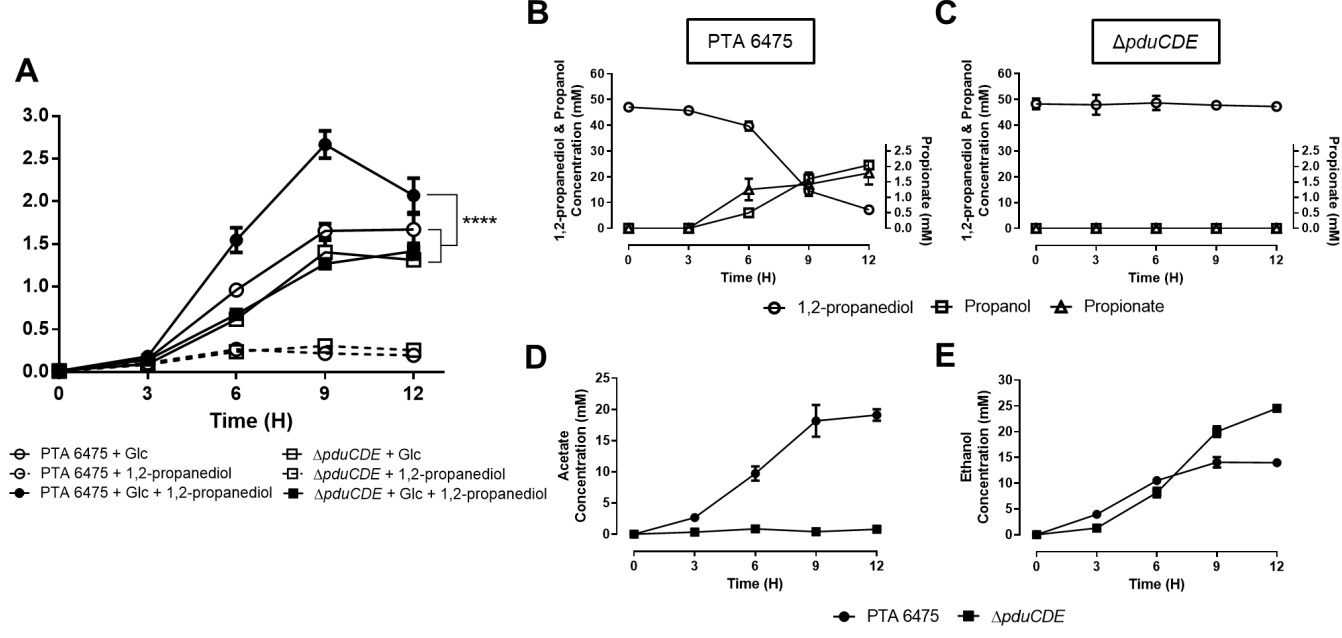
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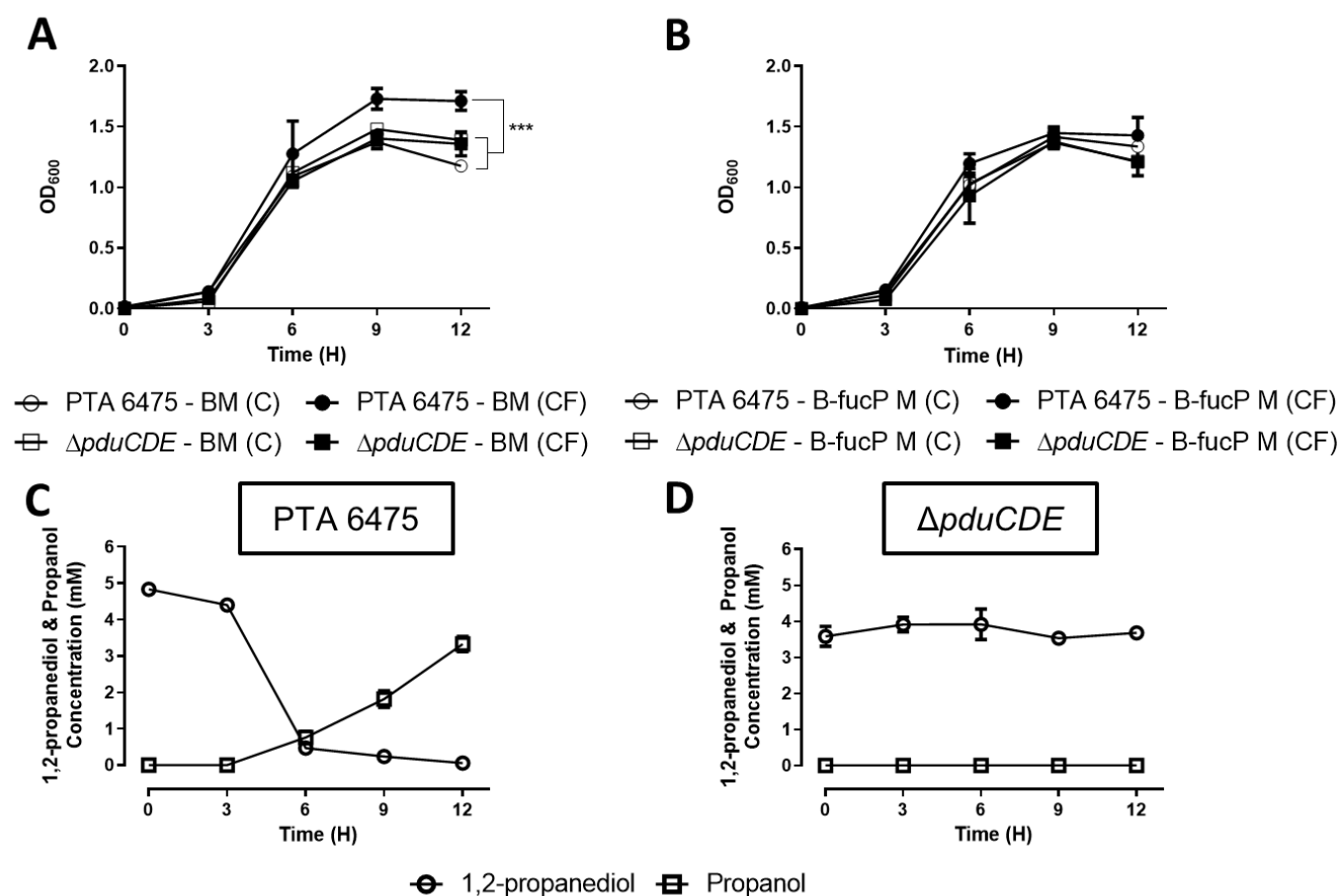
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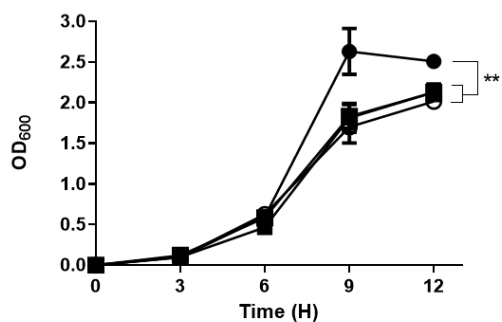
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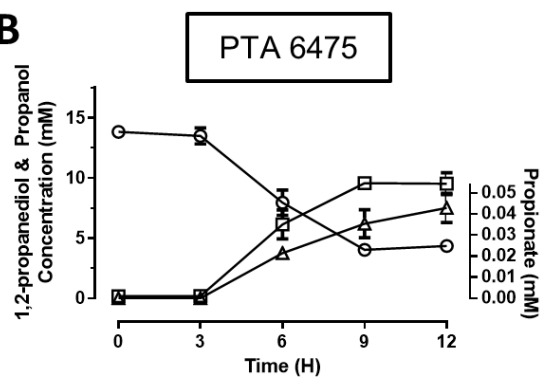


**A**



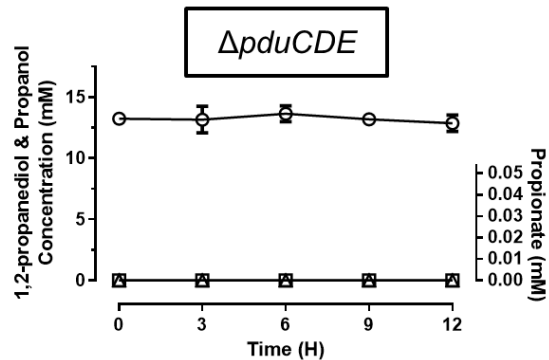
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□  $\Delta pduCDE$  - EM (G) ■  $\Delta pduCDE$  - EM (R)

**B**



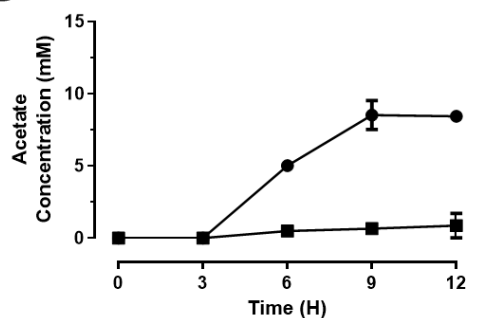
○ 1,2-propanediol □ Propanol ▲ Propionate

**C**



○ 1,2-propanediol □ Propanol ▲ Propionate

**D**



● PTA 6475 ■  $\Delta pduCDE$

